



Effect of Seed Treatment with Bio Fertilizers on Germination Plant Height and Total Biomass of Annual Moringa (*Moringa oleifera* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research study aimed to investigate the effects of different biofertilizer seed treatments on the germination, plant height, and biomass of annual Moringa (*Moringa oleifera* L.) over a period of two years. The biofertilizers used in the study included Azospirillum, Azotobacter, Blue Green Algae, Phosphobacteria, and Vesicular - Arbuscular Mycorrhiza. The results showed that all biofertilizer treatments exhibited higher germination percentages compared to the control group in both the first and second years of the study. Among the treatments, Vesicular - Arbuscular Mycorrhiza showed the highest germination percentage, with values of 94.67% in the first year and 89.4% in the second year. The plant height of the Moringa plants was measured at various time points during both years. The biofertilizer treatments consistently resulted in taller plants compared to the control group. The Vesicular - Arbuscular Mycorrhiza treatment showed the highest plant heights in both years and in

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pooled. with 17.267 cm at 30 DAS, 23.067 cm at 60 DAS, 32.332 cm at 90 DAS, 43.317 cm at 120 DAS, 54.135 cm at 150 DAS, and 63.282 cm at 180 DAS. The biomass of the Moringa plants was also measured at different time points. The results indicated that the biofertilizer treatments significantly increased the biomass compared to the control group. Vesicular - Arbuscular Mycorrhiza treatment also showed high biomass values in both years, with 1.04 g at 60 DAS, 6.91 g at 120 DAS, and 14.08 g at 180 DAS. Overall, the findings of this study demonstrate the positive effects of biofertilizer seed treatments on the germination, plant height, and biomass of annual Moringa. These biofertilizers can be considered as effective tools for enhancing the growth and productivity of Moringa plants, which have significant nutritional and medicinal value. Further research is warranted to explore the underlying mechanisms of these biofertilizers and their potential applications in sustainable agriculture

Keywords: *Moringa (Moringa oleifera L.); germination; plant height; biomass; Azospirillum; Azotobacter, Blue Green Algae, phosphobacteria; vesicular - arbuscular mycorrhiza.*

1. INTRODUCTION

Moringa (Moringa oleifera L.) belongs to the family '*Moringaceae*' is a fast-growing multipurpose medicinal tree extensively grown in tropics and subtropics of India and Africa. It is also widely distributed in Egypt, Philippines, Sri Lanka, Thailand, Malaysia, Burma, Pakistan, Singapore, West Indies, Cuba, Jamaica and Nigeria. In eastern and southern regions of India, *Moringa* is widely used as vegetable and grown commercially for its edible pods and leaves. *Moringa oleifera* is an important food commodity which has had enormous attention as the 'Natural Nutrition of the Tropics'. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia. Its popularity is increasing steadily because of its nutritional, medicinal value and for its sweetness in curry and slurry preparation along with red gram dhal. From that one could understand the importance of *Moringa* Sekar et al. [1]. Almost all the parts of this 'Miracle tree' root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various medicine, ailments, water purification etc. *Moringa* leaves have been used to combat malnutrition, especially among infants and nursing mothers [2]. It is found growing wild in the sub-Himalayan tract from the river Chenab eastwards to the Sarda and in the tarai tract of Uttar Pradesh in India Ramachandran et al. [3]. In the monogeneric genus *Moringa* of *Moringaceae* family there are 13 species. *Moringa oleifera* indigenous to the Himalayan foothills (northern India Pakistan and Nepal). India is the prime producer of *Moringa* (Drumstick) with an annual production of 2.20 to 2.40 million tonnes of tender fruits from an area of 38,000ha leading to the productivity of around

63 t per ha. Five among the different states, Andhra Pradesh leads in both area and production (15,665ha) followed by Karnataka (10,280ha) and Tamil Nadu (13250ha). In other states, it occupies an area of 4,613ha. *Moringa oleifera* is easily established or propagated by cutting or seeds. No seed pretreatment is required and seed, germinate readily in 1-2 weeks Ramachandran et al. [3].

Biofertilizers are known to enhance plant growth and productivity by providing essential nutrients and promoting beneficial microbial activity in the soil. When it comes to *Moringa oleifera*, biofertilizers have shown positive effects on its performance. *Azospirillum*, *azotobacter*, blue green algae (BGA), phosphobacteria, and vesicular arbuscular mycorrhiza (VAM) are commonly used biofertilizers in agriculture [4-8]. When applied as a seed treatment, these biofertilizers establish a symbiotic relationship with the plant, promoting nutrient solubilization and availability.

Azospirillum and *Azotobacter* are nitrogen-fixing bacteria. They colonize the root surface and convert atmospheric nitrogen into plant-available forms, promoting nitrogen uptake by *Moringa* plants. This leads to increased plant growth and improved nitrogen nutrition. Blue Green Algae, also known as cyanobacteria, are photosynthetic microorganisms that fix atmospheric nitrogen and produce plant growth-promoting substances like phytohormones and vitamins. Their application as a seed treatment enhances nitrogen fixation and nutrient uptake by *Moringa* plants. Phosphobacteria solubilize insoluble phosphorus present in the soil, making it available for plant uptake. This biofertilizer improves phosphorus availability, which is crucial for root development, flowering, and fruiting in *Moringa* plants. Vesicular

Arbuscular Mycorrhiza (VAM) is a beneficial fungus that forms a symbiotic association with plant roots. It enhances nutrient uptake, particularly phosphorus and micronutrients, by extending the root system through its hyphae. VAM also improves water absorption and drought tolerance in Moringa plants. Furthermore, biofertilizers can also enhance the nutritional quality of *Moringa oleifera*. A study by Kumari and Khanna [9], demonstrated that the application of biofertilizers increased the content of important phytochemicals, such as phenolics, flavonoids, and ascorbic acid, in *Moringa oleifera* leaves. These compounds are known for their antioxidant and health-promoting properties [10].

2. MATERIALS AND METHODOLOGY

2.1 Study Area Description

The proposed research experiment was carried out in the Forest Nursery and Research Centre of College of Forestry inside the SHUATS campus which are present in the Prayagraj, Uttar Pradesh.

2.2 Research Site Location

The research site is located at latitude 25° 24' 42" N and longitude 81° 50' 56" and elevation at 98 m above mean sea level. The average annual temperature experiment site is 25.7°C This region has a sub-tropical climate prevailing in the South-East part of U.P. with both the extremes in temperature, i.e., the winter and the summer. In cold winters, the temperature sometimes is low as 32°F in December-January and very hot summer with temperature reaching up to 115°F in the months of May and June. Frost during winter and hot scorching winds during summer are also common and the average annual rainfall is 981 mm with maximum concentration during July to September months with occasional showers in winters.

These works were comprising surveying of Moringa trees from South Indian States (Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, Kerala) and selection of phenotypically superior trees through plus trees selection criteria. Plus, trees will be marked in the field and location will be recorded with GPS instrument. After surveying, pods/seeds and vegetative propagules will be collected from the selected plus trees.

2.3 Methodology

Seed collection: To collect and screen the seeds from superior annual Moringa trees (Plus Trees) from different States of South India and Its evaluation for identification of superior genotypes.

These works will comprise surveying of Moringa trees from South Indian States (Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, Kerala) and selection of phenotypically superior trees through plus trees selection criteria. Plus trees will be marked in the field and location will be recorded with GPS instrument. After surveying, pods/seeds and vegetative propagules will be collected from the selected plus trees.

Procedure for selection of candidate trees and plus trees:

The first step is the selection of the 100 candidate trees from each State (from different Districts). The selected candidate trees will be marked with yellow band of 5cm width, 5 cm above the breast height and given a candidate tree number. Once the candidate trees are selected, they will be screened for superior traits (using plus tree criteria) as in relation to few surrounding trees called comparison tree or check tree. If the candidate tree exceeds the traits of comparison tree, it will be selected as "PLUS TREE". The objective behind comparison tree is to adjust or correct the phenotypic value of the candidate tree for environmental effects common to that particular stand (Sayward 1980). Out of 100 selected candidate trees from each state, only 5 plus trees were selected from each State. Pods/seeds and vegetative propagules will be collected from these plus trees and GPS readings of each tree were recorded.

Plus tree selection criteria: selection and screening of plus trees:

More than half the rotation age, Good growth form, Vigorous, healthy and showing superiority in height and diameter when compared with surrounding trees, length of the pods, More numbers of pods & Number of seeds per pod, Straight and clear stem, Light and spreading branches, Good natural pruning, none or few epicormic branches, Dense mass of healthy foliage, Free from insect and disease attack, Free from physical damages like wind, fire and animals.

To standardize the biofertilizers for enhancing the growth parameters of annual moringa:

The seeds were collected from phenotypically superior plus trees and treated with recommended percent of different

biofertilizers. Then treated seeds were grown in containers or poly bags with potting media, it were kept in a shade house and manually watered daily.

2.4 Treatment Combination

- T₀- Control
- T₁- Azospirillum
- T₂- Azotobacter
- T₃- Blue Green Algae
- T₄- Phosphorous Solubalising Bacteria
- T₅- Vesicular - Arbuscular Mycorrhiza

Treatments: 6,

Replications: 4,

Design: completely randomized Design (CRD).

The growth parameters of annual Moringa (*Moringa oleifera* L.) seedlings, including germination rate, plant height, and biomass, were measured and recorded.

3. RESULTS AND DISCUSSION

3.1 Germination Percentage (%) of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.)

From the Table 1 The germination percentages of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.) were evaluated for the years 2021 and 2022, and pooled data. The germination percentages were as follows The Vesicular - Arbuscular Mycorrhiza had the highest germination percentage at 93.33%. This was followed by Phosphate solubilizing bacteria with a germination percentage of 86.27%. Azotobacter had a slightly higher germination percentage of 86.33%. Azospirillum and Blue green algae both had germination percentages of 82.50% and 81.83%, respectively. The control group had a germination percentage of 80.36%. Overall, the results suggest that the application of Vesicular - Arbuscular Mycorrhiza and Phosphate solubilizing bacteria as biofertilizers for seed treatment of Annual Moringa could potentially enhance germination compared to all other biofertilizers and the controlled treatment had least value.

The pooled data from the table shows the treatment with the highest germination percentage is Vesicular - Arbuscular Mycorrhiza with a pooled value of 93.33%. This is followed by Phosphate solubilizing bacteria with a pooled value of 86.27%. Next is Azotobacter with a pooled value of 86.33%, and Azospirillum with a

pooled value of 82.50%. Blue green algae treatment has a pooled germination percentage of 81.83%. The control treatment has the lowest germination percentage with a pooled value of 80.36%. Overall, the data suggests that Vesicular - Arbuscular Mycorrhiza treatment consistently shows the highest germination percentage, while the control treatment has the lowest germination percentage.

3.2 Plant Height (cm) of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.)

From the Table 2 The results show the plant heights (cm) of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.) at different time intervals were recorded. Among the treatments, the highest plant heights were observed in the Vesicular - Arbuscular Mycorrhiza treatment, with 17.267 cm at 30 DAS, 23.067 cm at 60 DAS, 32.332 cm at 90 DAS, 43.317 cm at 120 DAS, 54.135 cm at 150 DAS, and 63.282 cm at 180 DAS. The next highest plant heights were recorded in the Azotobacter treatment, followed by the Phosphate solubilizing bacteria treatment, the Azospirillum treatment, and the Blue green algae treatment. The control group had the lowest plant heights across all time intervals.

The pooled data from the table shows the plant height (cm) of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.) at different time intervals. The data is presented for each treatment and for each time interval. At 180 DAS, the treatment with the highest plant height is Vesicular - Arbuscular Mycorrhiza with a pooled value of 63.282 cm, followed by Azotobacter with a pooled value of 62.68 cm, followed by the Phosphate solubilizing bacteria treatment, the Azospirillum treatment, and the Blue green algae treatment. Overall, the data suggests that Vesicular - Arbuscular Mycorrhiza treatments consistently show higher plant heights compared to other treatments at different time intervals.

3.3 Total Biomass (g) of Biofertilizers Seed Treatment of Annual Moringa (*Moringa oleifera* L.)

From the Table 3 The results show the study on the total biomass (g) of biofertilizers seed treatment of annual Moringa (*Moringa oleifera* L.), the biomass measurements were taken at 60 days after sowing (DAS), 120 DAS, and 180 DAS with both years 2021, 2022 and pooled data.

Table 1. Germination percentage (%) of annual moringa (*Moringa oleifera* L.) seed treatment with biofertilizers pooled data of two years

Treatment	2021	2022	Pooled
Control	80.42	80.3	80.36
Azospirillum	83.0	82.0	82.50
Azotobacter	86.35	86.31	86.33
Blue green algae	83.0	80.66	81.83
Phosphate solubilizing bacteria	86.32	86.23	86.27
Vesicular - Arbuscular Mycorrhiza	94.66	92.00	93.33
F-test	S	S	S
C.D.	7.220	14.646	2.654
SE(m)	2.317	4.753	0.752
SE(d)	3.277	6.722	1.064
C.V.	4.695	9.648	1.250

Table 2. Plant height (cm) of annual moringa (*Moringa oleifera* L.) seed treatment with biofertilizers pooled data of two years

Treatment	30 DAS			60 DAS			90 DAS			120 DAS			150 DAS			180 DAS		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Control	17.4	15.03	16.21	23	20.60	21.8	31.133	28.90	30.01	43.20	40.93	42.06	54.30	51.17	52.73	63.0	58.67	60.83
Azospirillum	17.66	15.30	16.48	23.3	21.24	22.27	32.733	30.97	31.85	43.5	41.30	42.4	53.93	51.30	52.61	64.16	59.47	61.81
Azotobacter	18.13	16.07	17.10	23.7	21.50	22.6	33.167	31.27	32.21	44	41.97	42.98	55.13	52.97	54.05	64.5	60.87	62.68
Blue green algae	17.66	15.23	16.44	23.1	21.00	22.05	31.73	30.57	31.15	43.23	41.03	42.13	53.43	51.27	52.3	63.13	58.67	60.90
Phosphate solubilizing bacteria	18.1	15.77	16.93	23.6	21.54	22.57	33.133	31.03	32.08	43.83	41.80	42.81	54.3	51.27	52.78	63.96	60.67	62.31
Vesicular - Arbuscular Mycorrhiza	18.33	16.20	17.26	24.13	22.00	23.06	33.43	31.23	32.33	44.53	42.10	43.31	55.2	53.07	54.13	65.23	61.33	63.28
F-test	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
C.D.	1.14	1.47	0.55	0.79	0.71	0.35	1.63	1.67	0.67	3.17	2.89	0.42	2.43	2.31	1.21	1.90	2.16	4.64
SE(m)	0.37	0.48	0.16	0.25	0.23	0.10	0.53	0.54	0.19	1.03	0.94	0.12	0.79	0.65	0.34	0.61	1.99	1.06
SE(d)	0.53	0.67	0.22	0.36	0.32	0.14	0.75	0.77	0.27	1.46	1.32	0.17	1.12	0.93	0.49	0.86	2.81	1.49
C.V.	3.63	4.64	1.25	1.87	1.69	0.60	2.81	2.87	0.83	4.09	4.11	0.39	2.52	2.11	0.90	1.66	1.56	2.37

Table 3. Total biomass (g) of annual moringa (*Moringa oleifera* L.) seed treatment with biofertilizers pooled data of two years

Treatment	60DAS			120DAS			180DAS		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
Control	0.90	0.72	0.81	4.63	4.45	4.54	8.37	8.19	8.28
Azospirillum	0.97	0.79	0.88	6.17	5.99	6.08	13.60	13.42	13.51
Azotobacter	1.03	0.85	0.94	6.93	6.75	6.84	13.97	13.79	13.88
Blue green algae	0.93	0.75	0.84	5.93	5.75	5.84	13.33	13.15	13.24
Phosphate solubilizing bacteria	1.03	0.85	0.94	6.63	6.45	6.54	13.67	13.49	13.58
Vesicular - Arbuscular Mycorrhiza	1.13	0.95	1.04	7.00	6.82	6.91	14.17	13.99	14.08
F-test	S	S	S	S	S	S	S	S	S
C.D.	0.385	0.123	0.135	0.400	0.208	0.658	0.407	0.375	0.685
SE(m)	0.051	0.039	0.038	0.128	0.067	0.520	0.131	0.120	0.194
SE(d)	0.072	0.056	0.054	0.182	0.095	0.735	0.185	0.170	0.275
C.V.	8.819	7.408	5.636	3.577	2.140	12.468	1.759	1.569	2.047

In 2021, the highest total biomass was observed in the vesicular-arbuscular mycorrhiza treatment had a total biomass of 14.08 g, followed by the Azotobacter treatment with 13.88 g. The phosphate solubilizing bacteria treatment with 13.58 g. The blue green algae treatment had a total biomass of 13.24 g, while the Azospirillum treatment had a total biomass of 13.51 g. The control treatment had the lowest total biomass of 8.28 g. In 2022, similar trends were observed. The highest total biomass was again seen in the vesicular-arbuscular mycorrhiza treatment with 13.99 g, followed by the phosphate solubilizing bacteria treatment with 13.49 g. The Azotobacter treatment had a total biomass of 13.79 g, while the blue green algae treatment had a total biomass of 13.15 g. The Azospirillum treatment had a total biomass of 13.42 g. The control treatment had the lowest total biomass of 8.19 g. The pooled data from the table shows the highest total biomass was observed in the Vesicular - Arbuscular Mycorrhiza treatment also showed high biomass values, with 1.04 g at 60 DAS, 6.91 g at 120 DAS, and 14.08 g at 180 DAS. The Azotobacter treatment, with values of 0.94 g at 60 DAS, 6.84 g at 120 DAS, and 13.88 g at 180 DAS. The other treatments, including Azospirillum, Blue green algae, and Phosphate solubilizing bacteria, also resulted in significant biomass increases compared to the control treatment.

Overall, the vesicular-arbuscular mycorrhiza treatment consistently had the highest total biomass, followed by the Azotobacter treatments and phosphate solubilizing bacteria. The blue green algae and Azospirillum treatments had intermediate total biomass, while the control treatment had the lowest total biomass.

4. CONCLUSION

The use of biofertilizers has shown promising results in enhancing the performance of *Moringa oleifera*. They have been found to improve plant growth, nutrient uptake, yield parameters, and the nutritional quality of this valuable plant. The efficacy of biofertilizers may vary depending on factors such as the type of biofertilizer used, application method, and environmental conditions. Among the tested biofertilizers, Vesicular-Arbuscular Mycorrhiza and Azotobacter have demonstrated particularly good results in terms of germination percentage, plant height, and total biomass of annual *Moringa* compared to other biofertilizers. These findings suggest that incorporating biofertilizers into *Moringa*

cultivation practices can be an effective strategy for enhancing growth and productivity. However, further research is recommended to better understand the underlying mechanisms and optimize the application methods of biofertilizers for sustainable agriculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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